



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61L 27/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/44965</b> <b>(43) International Publication Date:</b> 15 October 1998 (15.10.98)
<b>(21) International Application Number:</b> PCT/GB98/01017 <b>(22) International Filing Date:</b> 6 April 1998 (06.04.98)  <b>(30) Priority Data:</b> 9706967.8                      5 April 1997 (05.04.97)                      GB 9712399.6                      14 June 1997 (14.06.97)                      GB  <b>(71) Applicant (for all designated States except US):</b> GILTECH LIMITED [GB/GB]; 12 North Harbour Estate, Ayr KA8 8AA (GB).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> HEALY, David, Michael [IE/GB]; Midton House, Alloway, Ayr KA7 4EG (GB).  <b>(74) Agent:</b> MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> IMPLANTATION COMPOSITION COMPRISING GLASS PARTICLES		
<b>(57) Abstract</b> <p>There is provided a composition suitable for implantation in soft tissue (for example at or around a body orifice) in order to augment the volume of soft tissue. The composition described comprises particles of a, preferably water-soluble, biodegradable glass in a suitable carrier medium, such as glycerol. The particles, which are desirably irregularly shaped, may have an average particle diameter of from 50 <math>\mu\text{m}</math> to 2000 <math>\mu\text{m}</math>, preferably 50 <math>\mu\text{m}</math> to 300 <math>\mu\text{m}</math>. By injecting the particles into soft tissue, for example the bladder submucosa, it is possible to bulk up the soft tissue where this is required. This procedure can be applied to treat conditions such as vesicoureteric reflux. Additionally the procedure could be used cosmetically.</p>		

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1     **IMPLANTATION COMPOSITION COMPRISING GLASS PARTICLES**

2

3     The present invention is concerned with a composition  
4     suitable for implantation at or in the vicinity of a  
5     body orifice or sphincter muscle to aid correct  
6     function.

7

8     Many body functions rely upon the correct functioning  
9     of sphincter muscles. For example, the pyloric  
10    sphincter controls when the contents of the stomach  
11    pass into the small intestine. Similarly, the urethral  
12    sphincter controls when the contents of the bladder are  
13    voided. Incorrect functioning due to premature  
14    relaxation of such sphincter muscles can be  
15    problematic, and in the case of stress urinary  
16    incontinence (malfunction of the urethral sphincter)  
17    highly distressing to the patient.

18

19    Premature relaxation of a sphincter muscle often occurs  
20    when the sphincter muscle itself lacks sufficient bulk  
21    to adequately close the orifice in question. One  
22    option to overcome the problem is by implanting bulking  
23    material in the submucosa surrounding the orifice,  
24    thereby reducing the area to be closed by the sphincter  
25    muscle. Generally, the bulking material is injected

1 into the site to augment the soft tissue present.  
2 Suitable bulking materials are available commercially  
3 and are generally in the form of spherical particles or  
4 beads based on silicone, PTFE or collagen. These beads  
5 are suspended in a carrier fluid such as glycerine or  
6 hydrogel. The carrier fluid acts as a lubricant during  
7 the implantation process and assists expulsion of the  
8 implant from the syringe through an endoscopic needle.  
9 The carrier fluid is eliminated from the body and the  
10 implant material gradually becomes encapsulated by  
11 collagen at the implant site. The collagen capsule  
12 which forms around the implanted material adds to the  
13 bulk at the site. One such bulking material is  
14 MACROPLASTIQUE (Trade Mark) of Uroplasty, Inc.  
15

16 Existing implants do not biodegrade but remain  
17 permanently in the body of the patient. Recently,  
18 concern has been raised that such implants may  
19 gradually migrate away from the site of implantation  
20 during the lifetime of the patient. Thus, the original  
21 problem may recur as the size of the implant gradually  
22 decreases due to migration of the beads inserted. The  
23 patient will therefore need to undergo a further  
24 procedure in order to insert more beads at the site  
25 concerned. The migrating implant may, in addition,  
26 cause irritation and such implants have been reported  
27 to be associated with cancer, auto-immune and  
28 connective tissue disease.  
29

30 In addition to stress urinary incontinence, such  
31 implants have also been used to prevent vesicoureteral  
32 reflux. Vesicoureteric reflux is a condition occurring  
33 in babies and small children where the ureteral orifice  
34 is incompletely closed during contraction of the  
35 bladder. Urine is thus allowed to reflux back up the  
36 ureter and can cause recurrent infections of the

1 kidneys, frequently leading to permanent kidney damage.  
2 In a similar manner to stress urinary incontinence, it  
3 is possible to insert pellets or beads of silicone  
4 rubber or teflon in the submucosa of the bladder wall  
5 close to the ureteral orifice. Again, the procedure  
6 requires the permanent insertion of the implant.

7  
8 Paediatric vesicoureteral reflux usually resolves  
9 itself as the bladder wall thickens. By the time a  
10 child is five years old the urinary system has usually  
11 matured sufficiently to make the implant material  
12 redundant. Again, it is possible for implant material  
13 to migrate from the implant site causing obstruction,  
14 occlusion or embolism at another site. Implants have  
15 also been associated with cancer, auto-immune and  
16 connective tissue disease.

17  
18 The present invention provides a composition suitable  
19 for implantation in soft tissue (for example at or  
20 around a body orifice) in order to augment the volume  
21 of soft tissue. The composition of the present  
22 invention comprises particles of biodegradable glass in  
23 a suitable carrier medium. The carrier medium is  
24 required to ensure easy injection at the site of  
25 interest.

26  
27 The currently available silicone, PTFE and collagen  
28 beads are all deformable. This property aids injection  
29 of the beads, but also contributes to their ability to  
30 migrate from the site of interest. By contrast, the  
31 glass particles of the present invention are non-  
32 deformable.

33  
34 The composition is suitable for insertion in the  
35 bladder submucosa to treat stress urinary incontinence  
36 or vesicoureteric reflux by bulking up the area around

1 the urethral sphincter or urethral orifice  
2 respectively.

3

4 Optionally, the glass particles dissolve over a  
5 relatively long period, typically one to five years,  
6 more usually one to two years.

7

8 Preferably, the glass particles are irregularly shaped.  
9 This contrasts to the commercially available implants  
10 which are formed from spherically shaped beads. The  
11 irregular shape of the glass particles encourages their  
12 encapsulation in fibrous tissue. Such encapsulation  
13 further reduces the rate of dissolution of the glass  
14 and also helps to prevent migration of the particles.

15

16 Typically, the glass particles used in the present  
17 invention may have a diameter of from 50  $\mu\text{m}$  up to 2000  
18  $\mu\text{m}$ . More conveniently, however, the average diameter  
19 of the particles will be 1000  $\mu\text{m}$  or less, usually 500  
20  $\mu\text{m}$  or less. Good results have been obtained with  
21 particles having an average diameter of 300 to 200  $\mu\text{m}$   
22 or less, for example 150  $\mu\text{m}$  or less.

23

24 Particles having smaller diameters, e.g. 100  $\mu\text{m}$  or  
25 less, particularly of approximately 50 $\mu\text{m}$ , or even less,  
26 are of especial interest.

27

28 One advantage of the present invention is that it is  
29 possible to form glass particles having such small  
30 diameters (e.g. 50-100  $\mu\text{m}$ ). Where such small particles  
31 are used the problems associated with injection are  
32 reduced. Additionally, once the particles have been  
33 located in the site of interest, the outside surfaces  
34 of the particles becomes tacky as the particles begin  
35 to dissolve into body fluids so that the particles  
36 become associated in situ in a sticky cohesive mass.

1 Such particle association greatly reduces the rate of  
2 particle migration and the health risks associated  
3 therewith. No such association has been observed with  
4 the prior art silicone, PTFE or collagen beads.

5  
6 A carrier medium is generally used to assist injection  
7 of the particles. The carrier medium is typically  
8 glycerol, but other conventional carrier mediums (e.g.  
9 corn oil, sesame oil, sunflower oil or olibas oil) may  
10 also be used. A surfactant and/or suspending agent may  
11 also be included in the composition. Typical  
12 surfactants include, for example, benzyl benzoate,  
13 ethyl oleate and benzyl alcohol. Typical suspending  
14 agents include, for example, carboxymethylcellulose and  
15 alginate.

16  
17 In a further aspect the present invention provides a  
18 method of augmenting an area of soft tissue in a body  
19 (e.g. thickening a wall of a body organ), said method  
20 comprising injecting a composition into the soft tissue  
21 (e.g. the submucosa of said wall), said composition  
22 comprising particles of a biodegradable glass.

23  
24 Thus, the present invention provides a method of  
25 combatting vesicoureteric reflux by injecting a  
26 composition of the present invention into the bladder  
27 submucosa close to the ureteral orifice such that urine  
28 is substantially unable to pass up the ureter upon  
29 contraction of the bladder.

30  
31 Likewise, if the composition of the present invention  
32 is injected into the submucosa in the vicinity of the  
33 urethral sphincter, stress urinary incontinence may be  
34 overcome due to the "bulking" effect of the injected  
35 particles.

36

1 The present invention may be used at other body areas  
2 where soft tissue augmentation has a beneficial effect.  
3 Examples include injection around the anal passage, in  
4 order to reduce blood flow at the site and hence combat  
5 development of haemorrhoids (piles). Likewise soft  
6 tissue augmentation may be beneficial to temporarily  
7 correct an "incompetent" cervix which would prevent  
8 sustainment of a pregnancy. The soft tissue  
9 augmentation of the present invention may further be  
10 used to build up portions of the body damaged by  
11 accident or surgery, allowing healing to take place.  
12 Particular mention may be made of reshaping the facial  
13 area of a patient. From the above examples it is clear  
14 that the composition of the present invention may be  
15 used not only to treat existing conditions but also for  
16 prophylactic and cosmetic purposes.

17  
18 Generally the glass will be a controlled release glass  
19 (CRG). CRGs are vitreous inorganic polymers which  
20 dissolve over a pre-programmed period leaving virtually  
21 no residue. The components of manufacture are all  
22 present as natural body constituents hence CRGs show  
23 little or no cytotoxicity and exhibit a minimal tissue  
24 reaction.

25  
11 The use of glasses which can dissolve in water and body  
12 fluid are well-known. These glasses are formed from  
13 phosphorus pentoxide and may be modified to dissolve  
14 over a period of months or years, as required. To  
15 date, such glasses have been used, in medicine, for the  
16 controlled release of a number of agents, for example,  
17 drugs, hormones and trace elements.

18  
19 It is known that certain glasses, in which the usual  
20 glass former, silicon dioxide, of traditional glasses  
21 is replaced with phosphorus pentoxide as the glass



1 former, are soluble in water and body fluids. The rate  
2 of dissolution is controlled largely by the addition of  
3 glass modifiers such as calcium and magnesium oxide.  
4 In simple terms, the greater the concentration of the  
5 modifier the slower the rate of dissolution. The rates  
6 of dissolution which can be imparted to the glasses may  
7 range from minutes to months or even to several years.  
8 It is known to include in such compositions quantities  
9 of trace elements such as copper, cobalt and selenium  
10 which will be released from the glass as it slowly  
11 dissolves over the selected period of time.  
12

13 The use of water-soluble glasses has been described for  
14 a variety of purposes in the literature. For example,  
15 UK Patent Specifications Nos 1,565,906, 2,079,152,  
16 2,077,585 and 2,146,531 describe the gradual  
17 dissolution of the glasses as providing a means of  
18 controlled release of drugs, hormones, fungicides,  
19 insecticides, spermicides and other agents with which  
20 the glasses have been impregnated. The glasses are  
21 used, for example, in the form of an implant or bolus.  
22

23 UK Patent Specification No 2,030,559 describes the use  
24 of selenium-impregnated water-soluble glass for  
25 providing controlled release of the selenium as a trace  
26 element into cattle and sheep, the glass being applied  
27 as a subcutaneous insert. UK Patent Specification  
28 No 2,037,735 also describes a subcutaneous implant of  
29 water-soluble glass, and in this case the glass is  
30 impregnated with copper; minor quantities of trace  
31 elements such as boron, arsenic, iodine, manganese,  
32 chromium, silver, gold and gallium may also be  
33 included.  
34

35 Water-soluble glass has also been proposed for use in

1 prosthetics, for example in UK Patent Specification  
2 No 2,099,702, and for use in anticorrosive paints, as  
3 described in UK Patent Specification No 2,062,612.  
4 Further the literature provides for the use of such  
5 glasses in the controlled release of ferrous and ferric  
6 ions into the human or animal body by ingestion or  
7 implantation of the glass (UK Patent Specification  
8 No 2,081,703), and for the use of glasses in the  
9 controlled release of ions such as lithium, sodium,  
10 potassium, caesium, rubidium, polyphosphate, calcium  
11 and aluminium to patients by inclusion of the glass in  
12 a drip feed line (UK Patent Specification  
13 No 2,057,420).

14  
15 Optionally the water-soluble glass may be a silver  
16 containing water-soluble glass. Advantageously the  
17 silver content may be introduced into the glass  
18 composition in the form of silver orthophosphate.

19  
20 Suitable glasses include, for example, the ARGLAES™  
21 glass of Giltech Limited.

22  
23 The glass may be adapted by the use of glass modifiers  
24 to give a sustained release of silver ions over a set  
25 period.

26  
27 In one embodiment the water-soluble glass comprises an  
28 alkali metal oxide  $M_2O$ , an alkaline earth oxide  $MO$ ,  
29 phosphorus pentoxide  $P_2O_5$  and silver oxide ( $Ag_2O$ ) or  
30 silver orthophosphate ( $Ag_3PO_4$ ).

31  
32 Most preferably, said glass contains not more than 40  
33 mole %  $M_2O$  or  $MO$ , not less than 10 mole %  $M_2O$  or  $MO$ , and  
34 not more than 50 mole % nor less than 38 mole %  
35 phosphorus pentoxide, optionally with the inclusion of

1 0.05 to 5.0 mole % silver oxide or orthophosphate.

2

3 Said alkali metal oxide may be sodium oxide ( $\text{Na}_2\text{O}$ ),  
4 potassium ( $\text{K}_2\text{O}$ ) or a mixture thereof; and said alkaline  
5 earth oxide may be calcium oxide ( $\text{CaO}$ ), magnesium oxide  
6 ( $\text{MgO}$ ), zinc oxide ( $\text{ZnO}$ ) or a mixture thereof.

7

8 The glass may also contain less than 5 mole % silicon  
9 dioxide ( $\text{SiO}_2$ ), boric oxide ( $\text{B}_2\text{O}_3$ ), sulphate ion ( $\text{SO}_4^{2-}$ ),  
10 a halide ion, copper oxide ( $\text{CuO}$ ) or a mixture thereof.

11

12 Typically the soluble glasses used in this invention  
13 comprise phosphorus pentoxide ( $\text{P}_2\text{O}_5$ ) as the principal  
14 glass-former, together with any one or more  
15 glass-modifying non-toxic materials such as sodium  
16 oxide ( $\text{Na}_2\text{O}$ ), potassium oxide ( $\text{K}_2\text{O}$ ), magnesium oxide  
17 ( $\text{MgO}$ ), zinc oxide ( $\text{ZnO}$ ) and calcium oxide ( $\text{CaO}$ ). The  
18 rate at which the silver-release glass dissolves in  
19 fluids is determined by the glass composition,  
20 generally by the ratio of glass-modifier to  
21 glass-former and by the relative proportions of the  
22 glass-modifiers in the glass. By suitable adjustment  
23 of the glass composition, the dissolution rates in  
24 water at 38 °C ranging from substantially zero to 25  
25  $\text{mg/cm}^2/\text{hour}$  or more can be designed. However, the most  
26 desirable dissolution rate  $R$  of the glass is between  
27 0.01 and 2.0  $\text{mg/cm}^2/\text{hour}$ . The water-soluble glass is  
28 preferably a phosphate glass, and the silver may  
29 advantageously be introduced during manufacture as  
30 silver orthophosphate ( $\text{Ag}_3\text{PO}_4$ ). The content of silver  
31 and other constituents in the glass can vary in  
32 accordance with conditions of use and desired rates of  
33 release, the content of silver generally being up to 5  
34 mole %. While we are following convention in  
35 describing the composition of the glass in terms of the

1 mole % of oxides, of halides and of sulphate ions, this  
2 is not intended to imply that such chemical species are  
3 present in the glass nor that they are used for the  
4 batch for the preparation of the glass.

5  
6 The glass may be formed by a number of methods. It may  
7 simply be cast by conventional or centrifugal  
8 procedures, or it may be prepared via one or more  
9 stages of rod, fibre or tube drawing. Other  
10 preparation techniques include foamed glass. Following  
11 glass formation it will be comminuted into finely  
12 divided form.

13  
14 Optionally, the composition of the present invention  
15 may contain an active ingredient. The term "active  
16 ingredient" is used herein to refer to any agent which  
17 affects the metabolism or any metabolic or cellular  
18 process of the patient (including growth factors and  
19 living cells), promotes healing, combats infection,  
20 hypergranulation or inflammation. Antibiotics and  
21 other anti-bacterial agents, steroids, painkillers etc  
22 are all suitable. Optionally, the active ingredient  
23 may be in delayed-release or controlled-release form.

24  
25 The invention will now be further described with  
26 reference to the following, non-limiting, examples and  
27 Figures in which:

28  
29 Fig. 1 H and E staining of 1240596-1 glass granule  
30 intramuscular (six months). Magnification x 125.

31  
32 Fig. 2 H and E staining of 1240596-2 glass granule  
33 intramuscular (six months). Magnification x 125.

34  
35 Fig. 3 H and E staining of 1240596-3 glass granule

1 intramuscular (six months). Magnification x 125.

2

3 Fig. 4 Neutrophil staining of muscle section  
4 containing implant 1240596-1. Magnification x 125  
5 (black circles are air bubbles).

6

7 Fig. 5 Macrophage staining of muscle section  
8 containing implant 1240596-2. Magnification x  
9 125.

10

#### 11 Example 1

12

13 The CRGs will be implanted *in vivo* to assist in the  
14 evaluation of attenuation of the solution rate of the  
15 glass and to observe the acute tissue reaction at the  
16 submucosal implant site.

17

#### 18 Materials

19 Two CRG compositions with slow solution rates (to be  
20 decided) will be prepared as rough granules 200-300 $\mu$ m  
21 in diameter. The granules will be suspended in  
22 glycerine BP, 8.5 ml glycerine to 10 g CRG. The  
23 suspensions will be packaged 2.5 ml in syringes. The  
24 syringes will be individually sealed in foil  
25 polyester/polyester pouches and sterilised by  $\gamma$   
26 irradiation.

27

#### 28 Method

29 The anterior bladder wall of the anaesthetised model  
30 (rabbit) is exposed and a small volume (0.5 ml) of CRG  
31 implant is injected into the submucosa on the left and  
32 right anterior bladder wall midway between the ureters  
33 and the neck of the bladder. The implant should create  
34 a small visible mound at the implant site. It is  
35 suggested that the CRGs used at the left and right

1 sites be of different solution rates, or that one of  
2 the sites contains an existing "control" implant  
3 material for comparison (eg MACROPLASTIQUE (Trade Mark)  
4 of Uroplasty, Inc ten animals would be required.

5  
6 Evaluation

7 By placing the implants in the anterior bladder wall,  
8 it should be possible to look at the implant on a  
9 weekly basis using ultrasound. In addition, two  
10 animals would be sacrificed at two weeks, one month,  
11 six months and twelve months.

12  
13 The ultrasound examinations should look at the implant  
14 material and any migration from the implant site should  
15 be reported. Acute fibrous capsule formation should be  
16 recorded. It may be possible to differentiate the CRG  
17 and its dissolution over the more prolonged terms.

18  
19 On sacrifice, tissue reactions and acute inflammation  
20 should be recorded. Fibrous capsule development should  
21 be noted and presence of CRG (and glycerol in early  
22 stages) quantified for each implant site. Samples of  
23 surrounding tissues should be removed for histological  
24 examinations.

25  
26 Results and Interpretations

27 An initial inflammatory response is anticipated at the  
28 implant site. It is hoped that a collagen capsule will  
29 form around the CRG granules. This capsule is expected  
30 to reduce the solution rate of the glass. It will be  
31 helpful to measure the attenuation of solution rate due  
32 to reduced fluid transport within the capsule. By one  
33 month surrounding tissue inflammations should have  
34 subsided and histology should show normal cell  
35 response. There should be no migration of the CRG

1 implant beads and the glycerol should be completely  
2 removed within the first two weeks.

3

4 In each sacrifice group there should be at least one  
5 "control" implant. The tissue response of the control  
6 should be compared with the CRG implant results.

7

8 Example 2

9 Materials and Methods

10 Controlled release glasses (CRGs) were formulated as  
11 follows:

	mole % concentrations		
	Na <sub>2</sub> O	CaO	P <sub>2</sub> O <sub>5</sub>
I240596-1	5	48	47
I240596-2	15	38	47
I240596-3	25	28	47

1 A granular diameter range of 53-1000  $\mu$ m was used for  
2 all CRGs.

3

4 0.1 g samples of the CRGs listed above were sterilised  
5 by dry heat (190°C for 3 hours) before implantation  
6 into black and white hooded Lister rats (Liverpool  
7 strain). Two samples were implanted into each animal.  
8 Three animals were employed at a time period of six  
9 months. The implants were placed bilaterally into a  
10 pocket created in the dorsa-lumbar muscle region of the  
11 animal. At the six month time of explantation, the  
12 implant and surrounding tissue was removed from the  
13 sacrificed animal and frozen immediately. The frozen  
14 sample was sectioned at 7  $\mu$ m in a microtome cryostat.  
15 Analysis of the implant/tissue site was performed by  
16 staining the sample sections for various cytokines. A  
17 haematoxylin and eosin (H and E) stain was carried out

1 on each of the six retrieved samples, as well as  
2 neutrophil and macrophage staining.  
3 Immunohistochemical staining for ED1, ED2, CD4, CD8,  
4 Interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-2, Major Histocompatibility  
5 Complex (MHC) class II,  $\alpha$ - $\beta$  and Anti- $\beta$  antigens have  
6 been completed. These stains allow the tissue response  
7 to the implant presence to be evaluated in the  
8 following manner:

9  
10 H and E Stains all viable cells and allows the  
11 tissue type and fibrous capsule to be  
12 easily identified by the characteristic  
13 structure shape of each tissue.  
14  
15 ED1 Recognises rat macrophages, monocytes  
16 and dendritic cells. Granulocytes are  
17 negative. The recognised antigen is  
18 predominantly located intracellularly,  
19 although some membrane expression  
20 occurs.  
21  
22 ED2 Recognises a membrane antigen on  
23 resident rat macrophages; monocytes,  
24 dendritic cells and granulocytes are  
25 negative. No other cell types but  
26 macrophages are positive for ED2, and it  
27 discriminates between thymic cortical  
28 (ED2+) and medullary macrophages (ED2-).  
29  
30 CD4 Expressed on most thymocytes and  
31 approximately two thirds of peripheral  
32 blood T cells. In humans and rats, CD4  
33 is expressed on monocytes and  
34 macrophages. CD4 is an accessory  
35 molecule in the recognition of foreign



1 antigens in association with MHC class  
2 II antigens by T cells.

3

4 CD8 Expressed on most thymocytes and  
5 approximately one third of peripheral  
6 blood T cells, which constitute the CD4  
7 negative cells. CD8 $\alpha$  is in all natural  
8 killer (NK) cells in the rat.

9

10 IL-1 $\beta$  Expressed by B cells, macrophages and  
11 monocytes and its mRNA is present in a  
12 number of cells including T cells. In  
13 addition to activating T and B  
14 lymphocytes, interleukin-1 (IL-1)  
15 induces several haematological and  
16 metabolic changes typical of host  
17 response to infection and injury. IL-1  
18 is an endogenous pyrogen, producing  
19 fever by its ability to increase  
20 hypothalamic prostoglandin. IL-1 also  
21 induces the release of several  
22 lymphokines, interferons and colony  
23 stimulating factors. With the exception  
24 of skin keratinocytes, some epithelial  
25 cells and certain cells in the central  
26 nervous system, mRNA coding for IL-1 is  
27 not observed in health in most other  
28 cells.

29

30 IL-2 More descriptively, T cell growth  
31 factor, has promise as an immune  
32 stimulant and an anti-tumour agent. IL-  
33 2 recognises activated rat T cells but  
34 not resting lymphocytes.

35

1	MHC Class II	Expressed by dendritic cells, B cells,
2		monocytes, macrophages and some
3		epithelial cells. Expression is
4		increased by interferon $\alpha$ which also
5		induces expression on fibroblasts,
6		epithelial and endothelial cells.
7		
8	$\alpha$ - $\beta$	Detects an $\alpha$ - $\beta$ T cell receptor.
9		
10	Anti- $\beta$	Directed at leucocytes. Also labels B
11		cells among thoracic duct lymphocytes
12		with little labelling in bone marrow and
13		none on thymocytes. Acts as an isotope
14		control.

15  
16  
17  
18  
19 Results and Discussion

20 The photographs in Figures 1-3 show H and E staining of  
21 the I2405961-3 implants respectively. As can be seen  
22 in these Figures, fibrous capsules have formed around  
23 each glass granule. Glass I240596-1 has the slowest  
24 solution rate as tested in-vitro, and this can be seen  
25 in Figure 1 also, as the sizes of the remaining glass  
26 granules in the rat muscle after six months are  
27 considerably larger compared to the other two glass  
28 compositions which both have faster solution rates  
29 (I240596-3 has the fastest solution rate in-vitro).  
30 The surrounding muscle tissue to the implant appears  
31 healthy. Figures 4 and 5 show photographs of  
32 neutrophil staining of implant section I240596-1 and  
33 macrophage staining of I240596-2 respectively. These  
34 photographs are typical of all the slides viewed, as  
35 all six sections contained insignificant neutrophil and

1 macrophage presence in the tissue. In the photograph  
2 of the neutrophil stained section, it can be seen that  
3 there are several mast cells near the implant site and  
4 throughout the tissue. This is expected in normal,  
5 healthy muscle tissue. The lack of macrophages and  
6 neutrophils indicates a lack of inflammatory response  
7 to the implant, showing that after a six month period,  
8 the glass granules appear to be accepted in-vivo.

9  
10 The cytokine staining of the above antigens were all  
11 negative, correlating with the absence of neutrophils  
12 and macrophages in the tissue sections. Cytokines are  
13 regulatory peptides that can be produced by virtually  
14 every nucleated cell in the body, such as lymphocytes  
15 and monocytes. Cytokines are generally not  
16 constitutively produced, but are generated in  
17 emergencies to contend with challenges to the integrity  
18 of the host. Cytokines achieve these ends by  
19 mobilizing and activating a wide variety of target  
20 cells to grow, differentiate and perform their  
21 functions. This means that cytokines are key mediators  
22 of immunity and inflammation. The insignificant  
23 staining of the above indicates the acceptance of the  
24 glass implant into the body and shows that the glass  
25 presence is not inducing any inflammatory reaction in-  
26 vivo.

## 27 28 Conclusion

29 All the sections stained and viewed after the six month  
30 period showed healthy, normal muscle tissue containing  
31 a fibrous capsule coated glass granule. Staining of  
32 various cytokines gave a negative result, indicating  
33 the absence of inflammatory responses of the muscle  
34 tissue with the glass presence after six months.

35

### Example 3

#### Soft Tissue Response to Glycerol Suspended Controlled Release Glass Particulates

This example investigated the soft tissue response of glasses with a range of particulate sizes of different dissolution rates, transported in a glycerol carrier.

#### Materials and Methods

The CRG was tested in particulate form of three different compositions and two different particulate sizes: X (200-300 $\mu$ m, 0.02 mg/cm<sup>2</sup>/hr solution rate), Y (200-300 $\mu$ m, 0.12 mg/cm<sup>2</sup>/hr solution rate) and Z (<53 $\mu$ m, 0.34 mg/cm<sup>2</sup>/hr solution rate), all suspended in glycerol. A control sample of glycerol only was also included in the experiment and was labelled sample W. Samples weighing 0.1 grams of each of the CRG's in glycerol and glycerol only were sterilised by gamma irradiation before implantation intramuscularly into Wistar rats. Two samples were implanted into each animal. Four animals at each time period of 2 days, 4 weeks, 9 weeks and 6 months were employed. The implants were placed bilaterally into a pocket created in the dorso-lumbar muscle of the animal. At the time of explantation, the implant and surrounding tissue was removed from the sacrificed animal and snap frozen. A microtome cryostat was used to cut 7 $\mu$ m thick serial sections. Analysis of the implant/tissue site was performed by specific staining the sample sections for various cell types. Neutrophils and macrophages were stained using enzyme histochemistry, ED1 (monocytes and immature macrophages), ED2 (mature tissue macrophages), CD4 (helper/inducer T-lymphocytes and macrophages), CD8 (suppressor/cytotoxic T-lymphocytes), interleukin-1 $\beta$ , IL-2 (activated T-lymphocytes), Major Histocompatibility Complex (MHC) class II (activated

1    macrophages and activated B-lymphocytes),  $\alpha$ - $\beta$  (T-  
2    lymphocytes) and CD45RA (B lymphocytes) antibodies have  
3    been used to immunohistochemically stain each sample.

4

#### 5    **Results and Discussion**

6    Positive staining for neutrophils was observed after 2  
7    day implantation with all of the materials. The  
8    neutrophils present were found in localised clusters  
9    near the implant site. However, neutrophils were not  
10   seen in the tissue sections of each of the implanted  
11   glasses or glycerol in the remaining time periods.  
12   Mast cells were present in all tissue samples, but it  
13   was noticed that an increased number of these cells  
14   were present in clusters near the implanted glass in  
15   sections containing glass X at 6 months, glass Y at 2  
16   days and 6 months and glass Z at 4 weeks and 6 months.  
17   Enzyme staining and immunohistochemical staining both  
18   confirmed the presence of macrophages in all sections  
19   at all time periods except glass X at 6 months. The  
20   neutrophil presence at 2 days in all sections suggest  
21   an acute inflammatory response. The absence of these  
22   cells however in the remaining time periods indicate  
23   that this acute inflammation is quickly resolved.  
24   However, the presence of macrophages in all samples at  
25   all time periods except X at 6 months indicate an  
26   ongoing chronic inflammatory response to the presence  
27   of the implanted material. With glass X however, this  
28   chronic inflammatory response appears to have been  
29   resolved at 6 months. With one material, glass Z,  
30   tissues necrosis in association with the glass at 4  
31   weeks and 9 weeks has been observed. This study  
32   demonstrates that particulate, degrading glass is  
33   stimulating an inflammatory response in soft tissue of  
34   time periods up to 6 months. It should be noted that  
35   very small particulate fast degrading glass is leading

1 to tissue necrosis and should be further considered for  
2 these applications. However, larger particulate,  
3 slower degrading materials are demonstrating effective  
4 potential for stress incontinence applications.

5  
6 EXAMPLE 3

7 Inflammatory Response to Controlled Release Glass

8 Samples of a range of compositions of Controlled  
9 Release Glasses (CRGs) in granular form were analysed  
10 for the soft tissue response to determine their  
11 biocompatibility.

12  
13 Materials and Methods

14 The CRG was tested in granular form (53-1000 $\mu$ m) of  
15 three different compositions: A (high in CaO, slow  
16 solution rate), B (medium solution rate) and C (low in  
17 CaO, fastest solution rate). Samples weighing 0.1  
18 grams of each of the CRG's were sterilized by dry heat  
19 (3hrs, 190°C) before implantation into black and white  
20 hooded Lister rats. Two samples were implanted into  
21 each animal. Three animals were employed at each time  
22 period of 2 days, 1 week, 4 weeks, 8 weeks and 6  
23 months. The implants were placed bilaterally into a  
24 pocket created in the dorso-lumbar muscle of the  
25 animal. At the time of explantion, the implant and  
26 surrounding tissue was removed from the sacrificed  
27 animal and snap frozen. The frozen sample was  
28 sectioned at 7 $\mu$ m thickness in a microtome cryostat.  
29 Analysis of the implant/tissue site was performed by  
30 using different staining techniques.

31 Immunohistochemical staining using ED1 (monocytes and  
32 immature macrophages), ED2 (mature tissue macrophages),  
33 CD4 (helper/inducer T-lymphocytes and macrophages), CD8  
34 (suppressor/cytotoxic T-lymphocytes), interleukin-1 $\beta$ ,  
35 IL-2 (activated T-lymphocytes), Major

1 Histocompatibility Complex (MHC) class II (activated  
2 macrophages and activated T-lymphocytes),  $\alpha$ - $\beta$  (T-  
3 lymphocytes) and CD45RA ( $\beta$ -lymphocytes) antibodies have  
4 been performed. A haematoxylin and eosin (H and E)  
5 stain was carried out on each of the retrieved samples.  
6 Neutrophil and macrophage enzyme staining was also  
7 performed.

#### 8 9 Results and Discussion

10 The tissue response to the range of CRG's can clearly  
11 be demonstrated as being different and dependant on the  
12 materials, involving neutrophils, macrophages and mast  
13 cells and not involving T or B lymphocytes.

14  
15 Localised clusters of neutrophils were observed after 2  
16 days implantation of each of the CRG's A, B and C.  
17 However, neutrophils were not seen in the tissue  
18 sections of each implanted glass in each of the  
19 remaining time periods.

20  
21 Mast cells were scattered throughout all tissue  
22 sections as expected, but it was noticed that an  
23 increased number of these cells were present in  
24 clusters near the implant in sections containing CRG A  
25 at 9 weeks and 6 months, and in CRG C at 2 days, 9  
26 weeks and 6 months.

27  
28 The most predominant cell type in all sections was the  
29 macrophage confirmed by both enzyme staining and  
30 immunohistochemistry. Macrophages were observed in all  
31 of the sections for all of the time periods and were  
32 positive for ED1, ED2 and MHCII antibodies. The  
33 presence of neutrophils at 2 days in all three glass  
34 compositions indicate that an acute inflammatory  
35 response has occurred. The absence of the neutrophils

1 at all subsequent time periods suggest that the acute  
2 inflammatory phase had resolved. However, the  
3 observation of macrophages throughout all time periods  
4 up to and including 6 months indicates continued  
5 stimulus by the materials of a chronic inflammatory  
6 phase response.

7



1     **CLAIMS**

- 2
- 3     1.    A composition suitable for implantation in soft  
4           tissue, said composition comprising particles of  
5           biodegradable glass in a carrier medium.  
6
- 7     2.    A composition as claimed in Claim 1 wherein the  
8           glass particles are irregularly shaped.  
9
- 10    3.    A composition as claimed in either one of Claims 1  
11          and 2 wherein said particles have a diameter of  
12          1000  $\mu\text{m}$  or less.  
13
- 14    4.    A composition as claimed in any one of Claims 1 to  
15          3 wherein said particles have a diameter of 300  $\mu\text{m}$   
16          or less.  
17
- 18    5.    A composition as claimed in any one of Claims 1 to  
19          4 wherein said particles have a diameter of 50  $\mu\text{m}$   
20          to 100  $\mu\text{m}$ .  
21
- 22    6.    A composition as claimed in any one of Claims 1 to  
23          5 wherein said carrier medium is glycerol.  
24
- 25    7.    A composition as claimed in any one of Claims 1 to  
26          6 wherein said carrier medium includes a  
27          surfactant and/or a suspending agent.  
28
- 29    8.    A composition as claimed in any one of Claims 1 to  
30          7 comprising glass particles formed from a  
31          controlled release glass.  
32
- 33    9.    A composition as claimed in any one of Claims 1 to  
34          8 comprising glass particles formed from a water-  
35          soluble glass.

- 1     10. A composition as claimed in any one of Claims 1 to  
2         9 comprising glass particles formed from a silver  
3         containing glass.  
4
- 5     11. Use of a composition as claimed in any one of  
6         Claims 1 to 10 for augmentation of soft tissue.  
7
- 8     12. Use as claimed in Claim 11 wherein said soft  
9         tissue is the submucosa of the urethral sphincter.  
10
- 11    13. A method of augmenting an area of soft tissue in a  
12         body, said method comprising injecting a  
13         composition as claimed in any one of Claims 1 to  
14         10 into the soft tissue.  
15
- 16    14. A method as claimed in Claim 13 which is used  
17         augment soft tissue for cosmetic purposes.  
18
- 19    15. A method as claimed in Claim 13 wherein said soft  
20         tissue is the submucosa of a wall of a body organ.  
21
- 22    16. A method of combatting vesicoureteric reflux by  
23         injecting a composition as claimed in any one of  
24         Claims 1 to 10 into the bladder submucosa close to  
25         the urethral orifice such that urine is unable to  
26         pass up the ureter upon contraction of the  
27         bladder.  
28  
29  
30

1 / 3

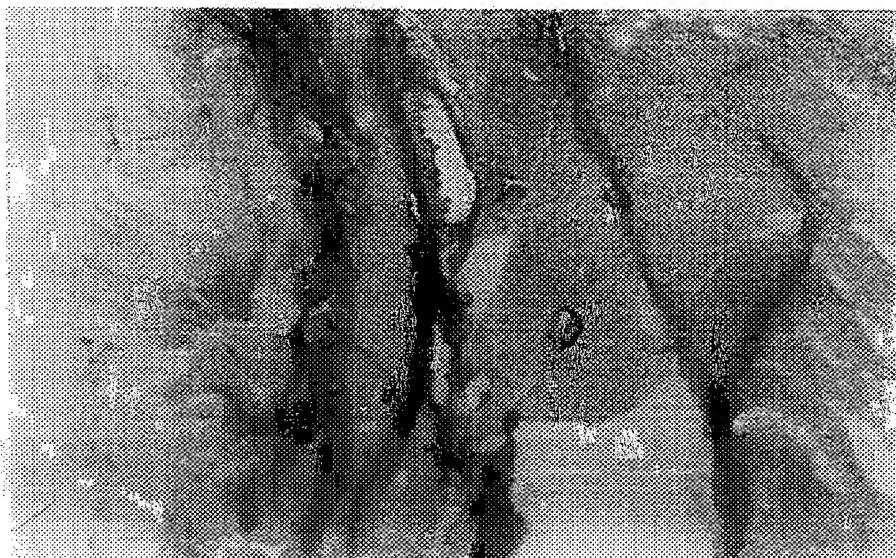


*Fig. 1*

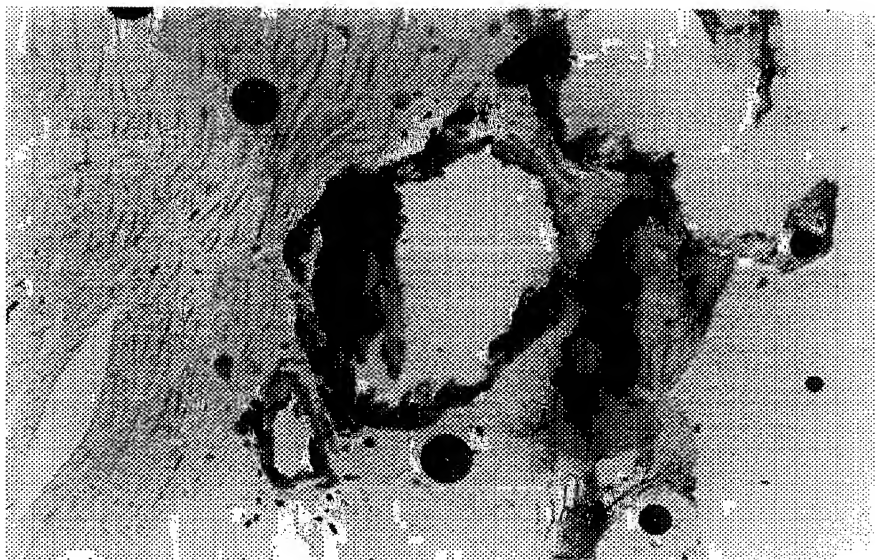


*Fig. 2*

2 / 3

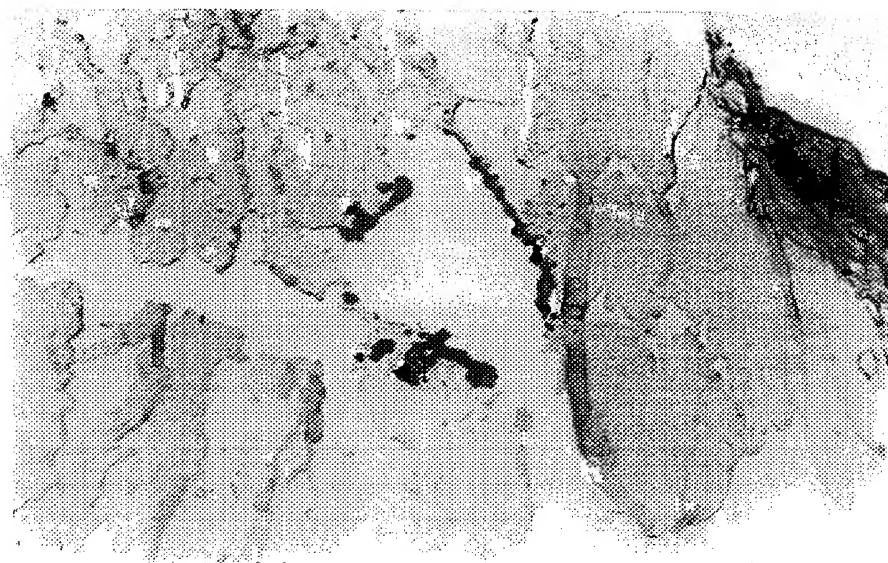


*Fig. 3*



*Fig. 4*

3 / 3



*Fig. 5*

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/01017

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61L27/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61L C03C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 204 382 A (WALLACE DONALD G ET AL) 20 April 1993 see column 7, line 36 - line 47; claims ---	1,3-6, 11-16
X	WO 93 16658 A (JSF CONSULTANTS LTD) 2 September 1993 see page 6, line 17 - line 28; claims ---	1,6,11, 12
Y	WO 93 15721 A (HUBBARD WILLIAM G) 19 August 1993 see claims ---	1-16
P,Y	WO 97 33632 A (GILTECH LTD ;GILCHRIST EILIDH (GB); GILCHRIST THOMAS (GB)) 18 September 1997 see claims; examples --- -/--	1-16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

4 August 1998

Date of mailing of the international search report

11/08/1998

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 98/01017

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	W0 90 08470 A (GILTECH LTD) 9 August 1990 ----	
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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 98/01017

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 13-16  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 13-16  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/01017

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Information on patent family members

International Application No

PCT/GB 98/01017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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